re-equilibration of a chemical system following an instantaneous increase in temperature induced by a laser pulse tuned to an infrared water band. The re-equilibration results in changes in the concentration of the species involved, and the transient changes are characterized using spectroscopic probes. To investigate the conformational changes associated with the binding of oxamate we studied the LDH from wild type cells as well as those from various single tryptophan mutants. These mutants were created by first replacing all tryptophans with tyrosine in wild type bsLDH to create a tryptophan-less template, followed by reintroduction of a single tryptophan at strategic sites in the protein. We probed the fluorescence emission of NADH in wild type and mutant bsLDH to report on the time evolution of the changes within the NADH environment over 100μs to 3ms time scale. Transients collected were then correlated to those resulting from a probe of tryptophan emissions. The results were then analyzed based on a plausible kinetic model. A comprehensive picture of the dynamics of ligand binding and Michaelis complex formation in bsLDH is obtained from the various structural reporters.

2914-Pos Board B19

Sequence, Structure and Dynamics Analysis of Thermostability in Endoglucanases

Ragothaman M. Yennamalli, Jeffrey D. Wolt, Andrew J. Rader, Taner Z. Sen.

Endoglucanases are crucial enzymes used in the production of biofuels from cellulosic biomass, a process which requires thermostability at high processing temperatures. Despite the economic importance of these industrial proteins, we currently lack a basic understanding of how some endoglucanases can efficiently function at elevated processing temperatures, while others with the same fold have substantial reduction in activity.

Here we explore the origins of thermostability in endoglucanases from sequence, structure, and dynamics perspectives using thermostable and mesostable protein sets. We performed a comparative sequence and structure analysis for thermophilic and mesophilic endoglucanases in $(\alpha/\beta)_8$, β-jelly roll, and $(\alpha/\alpha)_6$ folds, followed by a dynamics analysis of the $(\alpha/\beta)_8$ fold using elastic network models. We observed that thermophilic endoglucanases and their mesophilic counterparts differ significantly in their amino acid compositions. Interestingly, these compositional differences are specific to protein folds and enzyme families and lead to modification in hydrophobic, aromatic, and ionic interactions in a fold-dependent fashion.

We then focused specifically on a pair of thermostable and mesostable endoglucanases for a detailed dynamics analysis. It is often the case that thermophiles have shorter loops than their mesophilic counterparts, which was suggested to impart thermostability. In our case, however, the thermophile surprisingly possessed three insertions in the mesophilic loop regions and therefore has longer loops. The comparative structural dynamics analysis using elastic network models of $(\alpha/\beta)_8$ fold indicate that these three loops may contribute to the thermostability by modulating the direction of correlated motions between the catalytic residues (acid/base donor and nucleophile). We also observed that the thermostable protein showed larger dynamic domains than its mesostable counterpart, which suggests that cooperative dynamics is a critical contributing factor to thermostability.

2915-Pos Board B20

The Role of Dynamics in Protein Evolution

Tyler Glembo.

Protein evolution has most commonly been studied either theoretically, by analyzing the sequence of the protein, or experimentally, by resurrecting ancestral proteins in the lab and performing ligand binding studies to determine function. Thus far, structural and dynamic evolution have largely been left out of molecular evolution studies. Here we incorporate both structure and dynamics to elucidate the molecular principles behind the divergence in the evolutionary path of the gluccocorticoid and mineralocorticoid steroid receptor proteins. We begin by determining the likely structure of three evolutionary diverged, ancestral steroid receptor proteins using the Zipping and Assembly Method with FRODA (ZAMF). Our predictions are within 1.9Å RMSD of the crystal structure of ancestral corticoid steroid receptor. Beyond comparing static structure prediction, the main advantage of ZAMF is that it allows us to observe protein dynamics. Therefore we can investigate differences in the diverged proteins' available dynamic space by performing Principle Component Analysis (PCA) on the last .5ns of the converged MD trajectories obtained from ZAMF. We then analyze fluctuation profiles and cross-correlation maps from the slowest modes. This analysis enables us to identify critical mutations that most affect dynamics, therefore it shows the critical mutations leading to a divergence in function. We observe evolutionary diverged proteins do not share the same dynamic subspace. As this affects phenotype, we then compare binding specificities of these predicted structures to experimentally determined values by docking different ligands using ROSETTALIGAND and DrugScore online server.

2916-Pos Board B21

Conditional Mg^{2+}-Assisted Catalysis: A Master Switching Motif Responsible for Differential Stability Suggests a General Transducing Mechanism

Charles W. Carter, Violetta Weinreb, Li Li, Brian Kuhlman.

B. stearothermophilus Tryptophanyl-tRNA synthetase (TrpRS) uses different conformational states to catalyze tryptophan activation. A single Mg^{2+} ion increases transition state stabilization by −6.5 kcal/mol for optimal catalysis. Catalytic assist occurs if, and only if, the Mg^{2+} interacts with the protein. We are trying to identify the metal-protein interactions that produce this catalytic effect. Physical interactions between Mg^{2+} and TrpRS are mediated indirectly via active-site lysines K111, K192 and K195. Mutations of these lysines showed that they all stabilize the transition state. However, their interactions with the Mg^{2+} significantly reduce their catalytic effects. Catalytically productive interactions between TrpRS and the Mg^{2+} ion must therefore arise from outside the active site. We identified a core set of residues we call the D1 switch because they move during the catalytic conformational transition. The D1 switch lies at the corner of the N-terminal ß-α-ß crossover distal to the active site. It is highly conserved in Rossmannoid proteins. The Rosetta Design program suggested that mutations of D1 residues could ''hyperstabilize'' the activated state observed just prior to catalysis. Multimutant thermodynamic cycles together with substitution of Mn^{2+} for Mg^{2+} and [ATP]-dependent Michaelis-Menten kinetics demonstrate significant long-range *synergistic* coupling between the D1 switch and the Mg^{2+} ion. Thus, long-range interactions to the metal likely drive catalysis indirectly, by changing an inactive Mg^{2+} coordination into one that can stabilize the transition state. In this way transition-state stabilization by Mg^{2+} occurs if, and only if, conformational changes reposition it. We suggest that other NTPase enzymes may use similar conditional activation of Mg^{2+} to couple catalyzed hydrolysis of their purine triphosphate substrates to conformational changes, thereby transducing chemical free energy for cellular work and signaling. Supported by NIGMS 78227, 90406.

2917-Pos Board B22

Evolutionarily Conserved Linkage Between Enzyme Fold, Flexibility, and Catalysis

Pratul K. Agarwal.

Proteins are not static but rather are intrinsically flexible molecules. The role of internal protein motions in designated function, such as enzyme catalysis, is widely debated. The role of protein structure in enzyme catalysis is well established; and conservation of structural features provides vital clues to their role in function. Recently, it has been proposed that the protein function may involve multiple conformations: the observed deviations are not just inconsequential random thermodynamic fluctuations; rather, flexibility may be closely linked to protein function, including the catalytic efficiency of enzymes. We hypothesize that the argument of conservation of important structural features can also be extended to identification of protein flexibility in interconnection with enzyme function. Results from three classes of enzymes (prolyl-peptidyl isomerase, oxidoreductase and nuclease) catalyzing diverse chemical reactions will be presented. The identification and characterization of the internal proteins in multiple species show identical enzyme conformational fluctuations. In addition to the active-site residues, motions of protein surface loop regions are observed to be identical across species, and networks of conserved interactions/residues connect these highly flexible surface regions to the active-site residues that make direct contact with substrates. More interestingly, examination of reaction-coupled motions in non-homologous enzyme systems (with no structural or sequence similarity) that catalyze the same biochemical reaction show motions inducing remarkably similar changes in the enzyme-substrate interactions during catalysis. Examination of conformational sub-states along the reaction pathways also provides vital insights into role of enzyme flexibility in enabling the attainment of transition states. The results indicate that the reaction-coupled flexibility (along with structural features) is a conserved aspect of the enzyme molecular architecture. Protein motions in distal areas of homologous and non-homologous enzyme systems mediate similar changes in the active-site enzyme-substrate interactions, thereby impacting the mechanism of catalyzed chemistry.

2918-Pos Board B23

Probing the Kinetic Network of Folding-Unfolding Transitions in Proteins

Ronan D. Murphy, **Nicolae-Viorel Buchete**.

The formation of secondary and tertiary structure elements in protein folding are intrinsically complex processes, notoriously difficult to study in a systematic manner. We construct coarse master equations for helix formation processes based on data from atomistic molecular dynamics simulations of helix-rich proteins. By carefully controlling the effects of fast, non-Markovian transitions, on one hand, and the typically limited sampling of slow relaxation processes on the other hand, we probe the underlying network of folding-unfolding transitions between the various configuration states of a protein. This systematic analysis reveals the transition states and the

associated folding pathways at multiple levels, from atomistic to coarse-grained representations. We validate our approach in folding studies of short helix-forming polyalanine peptides, as well as of a larger, helix-turn-helix subdomain of a viral scaffolding protein. Our analysis of local, site-specific formation of intra- and inter-chain interactions is a first step towards understanding the elementary stages of secondary and tertiary structure formation in the folding of large proteins, and it allows a direct comparison to data from recent infrared vibrational spectroscopy studies.

2919-Pos Board B24

Flow-Induced Beta-Hairpin Folding of the Glycoprotein Ib-alpha Beta-Switch

Xueqing Zou.

Flow-induced shear has been identified as a regulatory driving force in blood clotting. Shear induces beta-hairpin folding of the glycoprotein Ib-alpha (GPIb-alpha) beta-switch which increases affinity for binding to the von Willebrand factor, a key step in blood clot formation and wound healing. To explore the mechanism underlying the flow-induced conformational transition, we conducted altogether 2.1 microsecond molecular dynamics simulations of flow acting on the beta-switch of GPIb-alpha. Simulations sampling different flow velocities reveal that under flow, beta-hairpin folding is initiated by hydrophobic collapse, followed by interstrand hydrogen bond formation and turn formation. Adaptive biasing force simulations are employed to determine the free energy required for extending the unfolded beta-switch from a loop to an elongated state. Lattice and freely-jointed chain models illustrate how the folding rate depends on the entropic and enthalpic energy, the latter controlled by flow. The results reveal that the free energy landscape of the beta-switch has two stable conformations, loop and hairpin, imprinted on it. Normal flow prefers the disordered state; high shear flow prefers the ordered state, inducing thereby a transition between the two.

2920-Pos Board B25

Protein Flexibility Partitions the Effects of Energy Landscape Roughness Between Activation Energy and Internal Friction

Anna A. Rauscher, Zoltan Simon, Gergely J. Szollosi, Laszlo Graf,
Imre Derenyi, Andras Malnasi-Csizmadia.

The rate of protein conformational changes are usually not only limited by external but also internal friction, however, the origin and significance of this latter phenomenon is poorly understood. By investigating the internal friction during the activation of two trypsin mutants at various temperatures and external viscosities we have discovered that the temperature dependence of the internal friction shows an Arrhenius-like behavior. The characteristic energy of the Arrhenius formula, however, can change dramatically upon the replacement of a single amino acid at a hinge position (thereby affecting the flexibility of the protein), or by crossing a critical temperature. At the same time, the activation energy of the conformational transition also changes with a similar magnitude, but in the opposite direction. These observations shed light on the intricate interplay between the apparent internal friction and activation energy. Moreover, we have found that the more flexible a protein is the greater proportion of its activation energy is partitioned into internal friction. All these results have allowed us to come to the general conclusion that the different hierarchical levels of the roughness of the energy landscape along a conformational transition can be observed as either activation energy or internal friction depending on the degree of flexibility of the protein.

2921-Pos Board B26

Relationship Between Internal Friction and the Roughness of the Energy Landscape of Protein Conformational Changes

Anna Á. Rauscher, Zoltán Simon, Gergely J. Szöllősi, László Gráf,
Imre Derényi, Andras Malnasi-Csizmadia.

We constructed a quantitative model based on experimental data that describes the relationship between the roughness of the energy landscape, activation energy and internal friction of enzyme conformational changes. We investigated an interdomain conformational rearrangement, trypsinogen 4 activation using transient kinetic methods. The temperature and viscosity dependence of the rate constant of the conformational change was measured in order to determine the temperature dependence of its internal friction. To test the effect of flexibility on internal friction, glycine and alanine mutations at a single position of the hinge of the interdomain region were introduced. Internal friction showed an Arrhenius-like temperature dependence, the characteristic energy of which increased with the flexibility of the hinge.

We found that the activation energy, i.e. the height of the energy landscape, is partially converted into internal friction to an extent depending on the flexibility of the protein. We interpret this phenomenon using a model that assumes different hierarchical levels of roughness of the energy landscape.

2922-Pos Board B27

Predicting Sequence of Events upon Ligand Binding Using PMT Model: A Case Study of Adenylate Kinase

Gamze Gursoy, Hsiao-Mei Lu, Jie Liang.

Conformational transitions play a crucial role during the reaction cycle of many enzymes. In the case of adenylate kinase (AK), binding of ATP and AMP induces a conformational change where the closing of the LID and NMP domains over the core domain is followed by the phosphate transfer from ATP to ADP. This conformational change is rate-limiting for the enzymatic reaction, and AK is thought to be in equilibrium between open and close states. In this work, we studied the sequence of events along the conformational transition pathway. Using the Perturbation-based Markovian Transmission (PMT) model [Lu and Liang, PLOS Computational Biology, 2009], we study each of the 45 intermediate conformations available in PDB. We apply an initial perturbation on the binding domains of the enzyme, whose transmission is modeled as a Markovian Process. The dynamics of the probability flow is then computed by solving the Master Equation using a Krylov subspace method. From the landscape of time-evolving probability flow of all residues upon initial perturbation, we calculated the information entropy and related parameter for each residue. By analyzing time-dependent changes in entropy of residues located within or are in-contact with the LID/NMP domains, we predicted contacts that would break first for each conformation. Using the initial open state conformation only we are able to identify the next conformation along the conformation transition pathway with an average accuracy of 85% in predicted bond breakage We also predicted a set of critical residues with distinct dynamic behavior that are important in ligand binding.

2923-Pos Board B28

A Comprehensive Examination of the Contributions to Binding and Activation Entropies

Jie Cao, Arieh Warshel.

The elucidation of the role of entropic effects in enzyme catalysis and binding free energy is a problem of practical and fundamental interest. In order to address this problem it is essential to develop simulation methods capable of evaluating the entropic contribution to the overall free energy. Such an evaluation is useful for assessing temperature effects and exploring specialized options in enzyme design. In fact, the general ability to evaluate activation entropies of chemical reactions in solution has long been a challenge to computational chemists. Here we present what is probably the first microscopic evaluation of all of the relevant components to the relevant entropy, namely, configurational, polar solvation and hydrophobic entropies. All of these contributions are evaluated by the restraint release (RR) approach. In the case of binding entropies we found out major compensation effects in both the solvation and hydrophobic effect, and despite some overestimate, can provide very useful insight. Furthermore, exciting current use of our approach lead to the elucidation of the origin of the puzzling strong temperature dependence of the activation entropy in ADH. It is found that this effect does not reflect any dynamical factor, but rather the change in the polarization of the protein polar groups (plus water molecules) upon moving from the ground state to the transition state. This helps to resolve the long-standing question about the origin of the observed non linear Arrhenius plot.

2924-Pos Board B29

Structural Instability of the Active Site of T1 Lipase where Na^+-π Interaction is Replaced with Water-π Complex

Yohsuke Hagiwara, **Jiyoung Kang**, Masaru Tateno.

The cation-π interaction is one of the strongest noncovalent forces. However, its biological role has been unknown, since few structures containing cation-π interaction have been determined in biological systems. Matsumura, et al. determined the crystal strcture of T1 lipase and found interactions between Na^+ and the aromatic ring of Phe16 in the active site. However, this Na^+-π interaction remained to be discussed whether it really exists or not. To investigate structural stability of Na^+-Phe interactions, we performed molecular dynamics (MD) simulations of T1 lipase.It is well known that the current conventional force fields cannot estimate the cation-π interaction correctly, whereas *ab initio* calculations require huge computational costs for the MD simulations. Accordingly, we developed a novel scheme to calculate the interaction energy with a high accuracy compared with the CCSD(T) level, and with a low calculation cost compared with the force field calculations. The result of our calculations definitely showed that the large enthalpy gain of the Na^+-π interaction was required to preserve the catalytic core structure. Since experimental approaches could not dismiss the possible presence of water instead of Na^+ in the active site of T1 lipase, we also examined the effects of water by performing MD simulations. Our analyses revealed that the water-π complex was unstable and led to the collapse of the coordinated structure of the active site. Thus, we